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Expression of the CD46 antigen, and absence of class I MHC antigen, on the human oocyte and preimplantation blastocyst

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SUMMARY

Expression of CD46 and class I major histocompatibility complex (MHC) antigens by human oocytes and 6–8-day unhatched expanded preimplantation blastocysts has been studied by immunocytochemistry. The CD46 antigen, a cell surface complement regulatory protein, was expressed by unfertilized oocytes as well as strongly by both the inner cell mass and trophectoderm of preimplantation blastocysts. In contrast, class I MHC antigens were not usually expressed by either oocytes or blastocysts. These data support the concept that gametes and embryonic cells involved in fertilization and early implantation events, respectively, may be protected from immunological recognition or attack both by the lack of class I MHC antigens and by expression of the CD46 complement regulatory protein.

INTRODUCTION

CD46 is a human cell surface glycoprotein synonymous with (i) the trophoblast–leucocyte common (TLX) antigen, (ii) the HuLy-m5 leucocyte differentiation antigen, and (iii) membrane cofactor protein (MCP) of the complement system.[1] It is a C3b-binding complement regulatory protein expressed by nearly all cells except erythrocytes, but strongly expressed by placental (foetal) trophoblast and uterine (maternal) endometrial gland epithelium;[2-4] this suggests a role for CD46 in down-regulating complement activation at the foeto–maternal tissue interface during pregnancy.[1,5]

Evidence has accumulated for involvement of the complement system in reproductive processes. For example, (i) there is significant C3 production by endometrial epithelium that is increased further in endometriosis,[6,7] (ii) systemic complement depletion in female rabbits before mating may result in a higher proportion of eggs being fertilized,[8] (iii) complement activity can lead to sperm damage and acrosomal loss, but is normally inhibited by factors in seminal plasma,[9] (iv) acrosome-reacted human sperm express CD46 on their surface, unlike non-reacted fresh ejaculated sperm, and treatment with anti-CD46 antibody causes a significant decrease in their ability to facilitate hamster egg penetration,[10] and (v) human oocytes have been shown to express both the CR1 and CR3 receptors (D. J. Anderson, H.-A. Wang and R. M. Jack, presented at the Society for Gynecologic Investigation, St Louis, MO, March 1990).

Previous immunofluorescence studies on cells in suspension have not detected class I or II major histocompatibility complex (MHC) antigen expression on the surface of unfixed human oocytes and up to 8-cell polyploid preimplantation embryos.[11,12] The TLX (CD46) antigen has also been reported to be absent from the surface of human oocytes using the same technique,[1] whereas preimplantation embryos have not previously been studied. The present study has instead used acetone-fixed material (i) to confirm the previous report[11] that class I MHC antigens are not normally expressed by human oocytes, (ii) to demonstrate that 6–8-day preimplantation embryos also lack expression of class I MHC antigens, (iii) to show, in contrast to the previous report,[11] that there is expression of CD46 by unfertilized oocytes, and (iv) to demonstrate that preimplantation blastocysts strongly express the CD46 antigen.

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MATERIALS AND METHODS

Preparation of oocytes and blastocysts

Human oocytes and preimplantation blastocysts were obtained from the *in vitro* fertilization programme at the University Dept. of Obstetrics and Gynaecology, Royal Liverpool Hospital. Ovarian stimulation, oocyte retrieval (Day 0), fertilization and embryo culture procedures were performed using established protocols.[13,14] Unfertilized oocytes, identified by examination for pronuclei 16–18 hr after insemination, had been in culture for 2–3 days before use. Oocytes and embryos were cultured in Earle's balanced salt solution (EBSS; Gibco, Paisley, Renfrewshire) supplemented with 10% (v/v) heat-inactivated (HI) autologous serum. Day 6–8 unhatched expanded blastocysts (100–200 cells) developing from embryos surplus to therapeutic needs, as well as oocytes, were used for immunocytochemical studies with patients' informed consent. All protocols had been approved by the Interim Licensing Authority and the Royal Liverpool Hospital Ethical Committee.

Individual oocytes and blastocysts were washed twice in serum-free EBSS and twice in isotonic saline on precleaned cavity glass slides (BDH, Poole, Dorset) before being fixed in cold acetone for 15 min and allowed to air dry. Slides were stored individually covered in aluminium foil at −70° for up to 28 days before batch experimentation.

Monoclonal antibodies

The murine monoclonal antibodies (mAb) used in this study were as follows: W6/32 (Sera-Lab Ltd, Crawley Down, Sussex), an IgG2a mAb directed against a non-polymorphic framework determinant of human class I MHC antigens;[15] 2A1, an IgG1 mAb which also recognizes a monomorphic determinant on class I MHC antigens[16] (P. C. L. Beverley, personal communication); H316, an IgG1 mAb raised against isolated human placental syncytiotrophoblast microvillous plasma membrane vesicles and which specifically recognizes the CD46 antigen;[1,3,17,18] TRA. 2.10, an IgG1 mAb also specific for human CD46;[19] H317 and H315, IgG1 and IgG2a mAb, respectively, that recognize human placental alkaline phosphatase (PLAP) and, for H315, also germ cell alkaline phosphatase (GCAP);[17,20] H319, an IgM mAb which recognizes a ubiquitous antigen expressed on all human cells and tissues that have been studied[21] was included as a positive control. Hybridoma cell supernatants were used in all experiments, and supernatants from non-antibody-producing clones were used undiluted as negative controls.

Immunocytochemistry

Antigenic expression by fixed human oocytes and blastocysts was demonstrated by reactivity with monoclonal antibodies using a sensitive biotin-streptavidin immunoperoxidase staining kit (Histostain-SP, Zymed Labs, San Francisco, CA). Cryostat sections (8 μ) of normal human term and first-trimester chorionic villous placenta, post-fixed in cold acetone, provided immunohistochemical controls for antibody reactivity and specificity.[3,21] The blocking solution was 10% non-immune rabbit serum. The primary antibody was linked to the streptavidin–peroxidase conjugate by a biotinylated second antibody (rabbit anti-mouse IgG/A/M), and bound streptavidin–peroxidase activity detected using hydrogen peroxide and the chromogen aminoethylcarbazole which produces a red/brown coloured deposit on oxidation. Mayer's haemalum was used as a counterstain. Samples were permanently mounted in glycerol–polyvinyl alcohol aqueous (GVA) mounting medium (Zymed Labs). Cells were examined using a Leitz Dialux 20EB microscope and photomicrographs taken using a Leitz Orthomat camera system.

Unfixed oocytes were also surface stained by an indirect immunofluorescence method. Oocytes were incubated for 10 min on ice with blocking buffer [phosphate-buffered isotonic saline (PBS), pH 7·4, with 0·2% BSA, 0·02% sodium azide, 10% HI-human AB$^+$ serum and 5% HI-rabbit serum], followed by a 30-min incubation on ice with mAb in blocking buffer. Oocytes were washed three times in cold wash buffer (PBS, pH 7·4, with 0·2% BSA and 0·02% sodium azide) and incubated in a 1:100 dilution of fluorescein-conjugated rabbit anti-mouse Ig (Serotec, Kidlington, Oxon) in blocking buffer for 30 min on ice. Oocytes were washed and fixed using 40% formaldehyde vapour for 5 min at room temperature. Slides were air dried and mounted in an anti-quenching medium (Citifluor Ltd, London, U.K.).

RESULTS

Human oocytes and preimplantation blastocysts clearly express the CD46 antigen, as demonstrated by immunocytochemical staining with the H316 and TRA.2.10 mAb compared with negative controls (Table 1, Fig. 1a and b). In contrast, neither oocytes (including the outer zona pellucida) nor blastocysts usually stained with mAb directed against class I MHC antigens (Table 1, Fig. 1c and d). Two oocytes out of a total of 11, however, were atypical in that they stained strongly for class I MHC with the W6/32 mAb (Table 1); none of five oocytes, all obtained from different donors from those that provided W6/32-reactive oocytes, stained with 2A1 mAb. The length of *in vitro* culture and morphological appearance of these two W6/32-positive oocytes were no different from all those unreactive with the W6/32 and 2A1 mAb. The positive control mAb (H319) gave consistent strong staining of both oocytes and blastocysts, in contrast to either a non-Ig-producing hybridoma supernatant or mAb to PLAP/GCAP which were all consistently negative. As a further control, each mAb was also screened under identical conditions onto human placental villous tissue cryostat sections; class I MHC antigen was expressed by foetal stromal cells and endothelium but not by chorionic villous trophoblast, whereas CD46 was strongly expressed by syncytiotrophoblast, in agreement with published data.[3]

The cellular distribution of immunocytochemical staining for CD46 on preimplantation blastocysts showed that both the trophectoderm and the inner cell mass stained strongly. Whilst the zona pellucida of oocytes remained unstained and the cytoplasm reacted only weakly with anti-CD46 mAb, there was usually strongest positive staining for CD46 at the peripheral edge of the oocyte cytoplasm. In order to ascertain expression of the CD46 antigen at the oocyte surface, unfixed oocytes were also stained by indirect immunofluorescence using the TRA.2.10 mAb. The oocyte membrane appeared as a bright positive fluorescent ring using this method, with the zona pellucida being more faintly and diffusely stained.

DISCUSSION

We have shown that the human CD46 antigen, a cell surface complement regulatory protein, is expressed by both oocytes and preimplantation embryos. The only earlier study,[11] using indirect immunofluorescence to screen unfixed human oocytes, reported the absence of cell surface expression of both CD46 (TLX) and class I MHC antigens; blastocysts were not investigated. In order that we could screen oocytes and blastocysts with several mAb in batch experimentation, we have used mostly acetone-fixed cells. This would allow mAb to bind to cytoplasmic structures as well as the outer plasma membrane. However, additional cell surface immunofluorescence staining experiments using unfixed oocytes have confirmed that the oocyte plasma membrane stains for CD46.

The role of CD46 in complement regulation is to bind C3 fragments and serve as a membrane cofactor for proteolytic inactivation of C3 convertase by factor I.[22] The widespread tissue distribution and cell surface location indicate its major function in minimizing complement-mediated damage at autologous cell surfaces. In pregnancy, foetal trophoblast invasion into endometrial tissue following implantation occurs with concominant tissue damage and restructuring, as well as haemostatic alterations, that may be expected to cause persist-

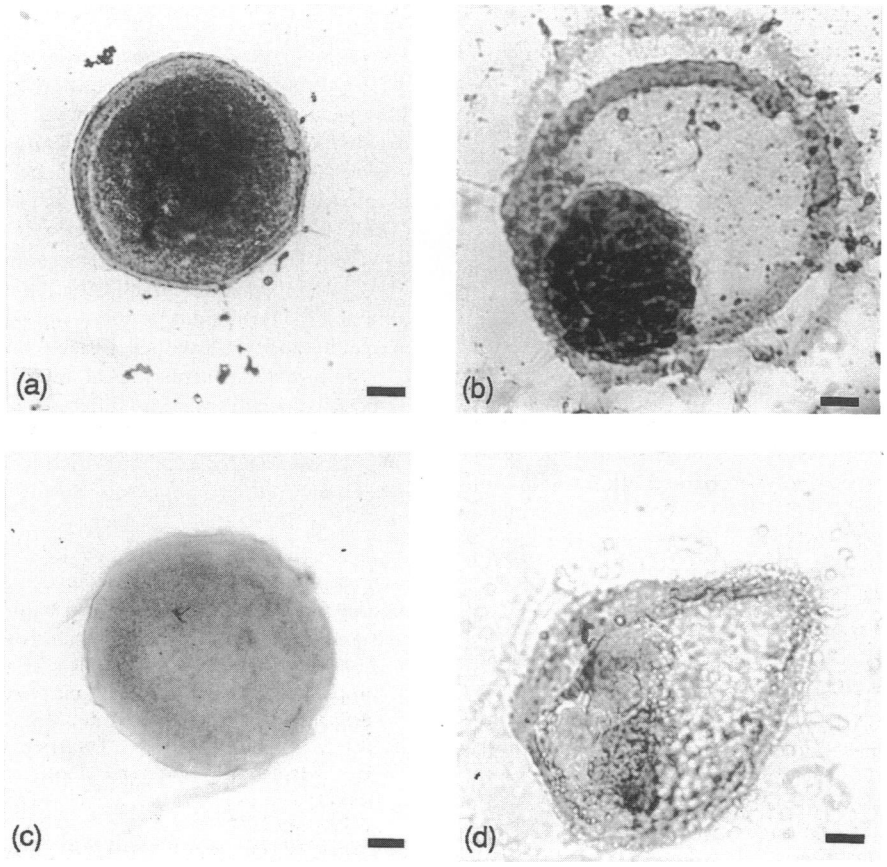

Figure 1. Detection of CD46 antigen using the H316 mAb on (a) a human oocyte and (b) an unhatched expanded blastocyst, and the lack of staining for class I MHC antigens using the W6/32 mAb on (c) a human oocyte and (d) an unhatched expanded blastocyst. All specimens were counterstained with Mayer's haemalum so that the immunoperoxidase reaction product is seen as the darker contrast stain present only in (a) and (b). Bar = 15 μm.

Table 1. Reactivity of monoclonal antibodies with human oocytes and preimplantation blastocysts

		Oocytes		Blastocysts	
Monoclonal antibody	Specificity	Staining intensity	No.	Staining intensity	No.
H316	CD46	+	6	++	4
TRA.2.10	CD46	+	6	++	3
W6/32	Class I MHC	−	9	−	3
		++	2		
2A1	Class I MHC	−	5	−	3
H319	Ubiquitous cell surface antigen	++	5	++	3
Non-Ig producing hybridoma supernatant	None	−	6	−	2
H317/H315	PLAP and, for H315 only, also GCAP	−	5	−	2

−, +, ++, Represent no staining, obvious staining, and consistent strong staining, respectively.

ent low-level collateral complement activation. Our data add further support to an important biological role for CD46 in down-regulating local tissue complement activation during blastocyst implantation. In addition, CD46 may have a role in complement-dependent processes during sperm–oocyte and blastocyst-endometrial epithelium cell interaction events. Both occur between genetically dissimilar cells that carry one or more C3-binding molecules, and it is of particular interest that sperm demonstrate surface expression of CD46 only after the acrosome reaction.[10]

The absence of human class I MHC antigen expression by 6–8-day preimplantation blastocysts, demonstrated in this study, substantially extends the only previous observations on 2–8-cell stage embryos.[12] In that report,[12] most embryos at such a very early stage may have been expected to express proteins under the control of the maternal genome since transcription from the embryonic genome first occurs only after the third cleavage division (4–8-cell stage).[23,24] The failure of blastocysts to present any class I MHC antigen is of obvious evolutionary advantage to their survival in order to abrogate T-cell or alloantibody recognition at a time when they may be highly vulnerable to immune rejection. In addition, as confirmed in the present study, it is of interest that both gametes do not usually express class I MHC antigens prior to fertilization.[11,25] Recent evidence has shown that a non-classical I MHC antigen, HLA-G, is expressed by differentiated invasive cytotrophoblast early in gestation,[26–28] and it was possible that the two oocytes in the present report that atypically stained with the W6/32 mAb could have reflected unusual expression of HLA-G or a similar antigen. However, this or other explanations could not be further explored since additional oocytes from the same donors were not available.

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